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[Continued on next page]

(54) Title: INJECTABLE CALCIUM SALT BONE FILLER COMPRISING CELLS



(57) Abstract: The invention relates to a bone filler comprising calcium salt particles, an organic binder having an affinity for calcium phosphate, cells chosen from the group of stem cells, osteogenic cells, and osteoprogenitor cells, and a pharmaceutically acceptable buffer. A great advantage of a bone filler according to the invention is that it is an injectable formulation that allows for its introduction in an osseous defect through the needle of a syringe.

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## INJECTABLE CALCIUM SALT BONE FILLER COMPRISING CELLS

The invention relates to the field of reconstructive surgery, and in particular to the repair of osseous defects in a patient.

Successful closure of bone defects remains a major concern to reconstructive surgeons. While most often secondary to trauma, bone loss can also arise from congenital disorders, neoplasms, and infections. A wide variety of materials have been employed to repair osseous defects, including autogenous cells, allogeneic tissues, and alloplastic materials. This variety of approaches attests to the absence of an optimal method for restoring bone integrity, especially in the presence of a sizable defect.

Aside from the selection of a suitable material for repair of an osseous defect, the reconstructive surgeon is also faced with the problem of accessibility. In order to be able to insert a reconstructive material at the site of the defect, it is often necessary to make considerable wounds, causing trauma and discomfort to the patient, since typically the location of the osseous defect is inside the body in the bone structure of the patient.

Conventional constructs used to repair osseous defects have a rigid, inflexible structure, as they must be able to take over the supporting tasks of living bone tissue. Also, they are of a size dependent on the size of the osseous defect, as the complete defect should preferably be repaired in one surgical operation. Hence, the larger the defect that is in need of repair, the greater the opening of the wound must be in order to be able to insert the construct into the defect.

In view of these circumstances, there is a need for a material that is suitable to function in the repair of bone tissue which has a flexibility that allows its introduction into the patient's body through a small wound opening, but nevertheless has such mechanical properties that enables it to assist in the supporting function of bone tissue, and preferably is ultimately converted into actual bone tissue.

US patent 6,129,761 discloses an injectable hydrogel composition comprising a hydrogel based on hyaluronic acid, a synthetically modified alginate, or another crosslinkable polymer capable of forming a hydrogel, and dissociated cells, such as bone cells, muscle cells, fibroblasts or organ cells. The  
5 composition is specifically intended for cartilage or organ repair.

The international patent application 95/21634 discloses a biomaterial for the resorption/substitution of supporting tissue, tooth substance, bony tissue or osteoarticular tissue. The composition is injectable and comprises an inorganic phase of calcium phosphate particles, and an  
10 aqueous solution of a cellulose-derived polymer. The calcium phosphate particles need to be either a mixture of tricalcium phosphate  $\beta$  and hydroxyapatite in a ratio of 20/80-70/30, or calcium-titanium-phosphate.

US patent 6,287,341 discloses a method for repairing an osseous defect wherein two calcium phosphates are mixed with a physiological liquid to  
15 provide a paste or putty which is applied to the osseous defect to harden at the implant site. The hardening occurs as a result of a reaction between the two calcium phosphates. It is mentioned that the paste or putty may comprise live cells, such as osteoblasts, osteoclasts, chondrocytes, osteocytes or fibroblasts. These cells, however, are not expected to be able to withstand the harsh  
20 conditions during the hardening of the paste or putty.

The international patent application 00/07639 discloses bone precursor compositions. A calcium cement is mentioned for being suitable for injection into a bone defect. The cement is based on monobasic calcium phosphate monohydrate and  $\beta$ -tricalcium phosphate, and may further  
25 comprise a biopolymer foam, collagen, an extracellular matrix component, a therapeutic agent, a biopolymer fibre, or live cells. After injection, the calcium cement require setting, which is likely to be harmful to any living cells present.

It is an objective of the present invention to provide a bone filler  
30 which can be used for tissue repair, which bone filler comprises cells, wherein

the risk of harm to the cells (e.g. due to setting of a calcium phosphate phase) is substantially avoided. The objective bone filler should have such properties that it can be easily processed and be injected into an osseous defect in a patient through the needle of a syringe under sterile conditions. It is further  
5 desired that the cells will not be substantially harmed by being injected through for instance a syringe. Other objects and advantages of the invention will become clear from the following description.

In accordance with the invention, an injectable bone filler is provided, which bone filler comprises calcium salt particles, an organic binder  
10 having an affinity for the calcium salt, cells chosen from the group of stem cells, osteogenic cells, and osteoprogenitor cells, and a pharmaceutically acceptable buffer.

A bone filler according to the invention is injectable, which means that it can be administered to the site of an osseous defect through injection.  
15 To this end, it is preferred that a syringe is employed. The bone filler has such flexibility that it can pass through the needle of a syringe. This has as a great advantage that only a very small wound needs to be made in order to introduce the filler at the desired location, which spares the patient a considerable discomfort and possible trauma.

20 Further, the presence of calcium salt particles in the bone filler allows for *de novo* bone formation *in vivo*. As a result, the filler is ultimately converted into autologous bone tissue and can assist in the supporting function of the bone in an early stage. Also, it was found that the calcium salt particles may function as a kind of seeding crystals *in vivo* on which additional calcium  
25 salt is deposited. Accordingly, the bone filler hardens and provides strength soon after implantation.

Surprisingly, it has further been found that living cells can be incorporated into the formulation of a bone filler according to the invention in such a manner that the bone filler can be injected without substantially  
30 negatively affecting the viability of the cells. In fact, the presence of the cells in

the bone filler have a significant positive impact on the rate at which bone formation occurs *in vivo* after administration of the bone filler (in)to an osseous defect.

As mentioned above, a bone filler according to the invention  
5 comprises calcium salt particles. Dependent on the location of an osseous defect that is to be repaired with the filler, the skilled person can suitably select a calcium salt. Possible choices are for instance monetite, brushite, (CaHPO<sub>4</sub>), calcium pyrophosphate, and calcium carbonate. Preferred is the use of calcium phosphate salts, in particular hydroxyapatite,  $\beta$ -calcium phosphate,  
10 and combinations thereof, such as in a mass ratio of 60/40. All of these materials occur naturally in living bone and are consequently readily accepted by a living organism. Particularly good results have been achieved using hydroxyapatite.

An important parameter of the calcium salt particles was found to  
15 be their particle size. Preferably, the particles have a diameter of from 100 to 600  $\mu\text{m}$ , more preferably of from 200 to 400  $\mu\text{m}$ . As is also shown in the appended examples, a relationship was surprisingly found between the size of the calcium salt particles and rate and extent of bone formation induced *in vivo*.

20 Calcium salt particles of the desired size can conveniently be prepared by crushing calcium salt and sieving at the right mesh size. It is preferred that a sintered calcium salt is used, which is optionally water tumbled before sintering to obtain a dense material. It is preferred that dense and smooth calcium salt particles are employed, as this significantly reduces  
25 the risk of inflammation *in vivo*.

Another important substance present in a bone filler according to the invention is the organic binder. The binder should have sufficient affinity for the calcium salt to allow the formation of a homogeneous paste to form the injectable bone filler. Further, it will be understood that the binder should be  
30 of a material that is acceptable for introduction into a living organism.

Preferably, the binder is biodegradable so that it disappears once the deposition of calcium salt and/or the bone formation has taken place to a sufficient extent to take over the function of living bone.

It is furthermore desired that the binder contributes to the viscosity of the bone filler. It serves on the one hand to keep the calcium salt particles together as to form a paste of sufficient integrity, and on the other hand to impart sufficient flexibility to the bone filler to allow for its administration through the needle of a syringe.

Suitable examples of materials that can be used as the organic binder in a bone filler according to the invention include alginates, dextrans, cellulose, derivatives of cellulose, plasma (blood plasma), biogenic binders, hyaluronic acid, and combinations thereof. Specific examples are sodium alginate, sodium carboxymethyl cellulose, dextran, fibrin glue, and transglutaminase. It is preferred to use sodium alginate as it was found that this binders allows for a very convenient formulation of the bone filler.

Dependent on the nature of the binder chosen, it is preferably present in a bone filler according to the invention in an amount ranging from 0.5 to 10 wt.%, more preferably from 3 to 7 wt.%, based on the weight of the bone filler.

Suitable cells that may be incorporated are stem cells, osteogenic cells, and osteoprogenitor cells. It is preferred that the cells that are incorporated into the bone filler are obtained through a biopsy from the patient to which the bone filler is ultimately to be administered, *i.e.* that autologous cells are used.

In order to assist in the formulation of a bone filler according to the invention, it is usually preferred to use and incorporate a buffer. The buffer can also serve to ensure that the osmolarity of a bone filler according to the invention is similar to the osmolarity in the surroundings of the osseous defect into which the bone filler is to be injected, thereby avoiding an undesired impact of the filler on living tissue at the site of implantation. Although in

principle any liquid that is sufficiently pharmaceutically acceptable can be used, it is preferred that a saline solution essentially not causing osmotic pressure to cells (usually around 8 g/L) and comprising a biocompatible buffer (preferably at a pH around 7.4) is employed. Especially preferred is the use of  
5 phosphate buffer saline (PBS) as buffer.

The amount of buffer used will depend on the viscosity of the chosen binder and the desired viscosity of the bone filler. Generally, the bone filler will be formulated to have a solids content of 30-70 wt.%, preferably 40-60 wt.%.

10 In order for a bone filler according to the invention to pass through a needle of a syringe without great difficulty, its Brookfield viscosity will generally lie between 30,000 and 100,000 centipoises.

In the preparation of a bone filler according to the invention, it has proven to be of advantage to first prepare a gel of the organic binder and the  
15 buffer. To this end, the binder is mixed with or dissolved in the buffer. Preferably, and depending on the binder, care is taken during mixing that the binder does not form agglomerates. To the prepared gel, the calcium salt particles can be added and they can be mixed to form a homogeneous paste, being the objective bone filler.

20 In a preferred embodiment, cells are seeded onto the calcium salt particles before they are added to the gel formed by the organic binder and the buffer. It is also possible to introduce the cells after the calcium salt particles, the organic binder and the buffer are brought together. In the latter embodiment, it is possible that the cells actually adhere to the calcium salt  
25 particles prior to injection of the filler into a patient, but it is also possible that they will be part of the injectable bone filler as a separate component. If the cells adhere to the calcium salt particles, it can be said that the particles are coated with cells.

The seeding of the cells to the calcium salt particles can be carried  
30 out in any conventional manner. Preferably, the cells are cultured for one or



more passages before the calcium salt particles carrying the cells are formulated together with the gel formed by the organic binder and buffer. The culturing is preferably performed under dynamic conditions, *e.g.* as described in European patent application 1 002 859, in order to retain sufficient fluidity.

5 During the culturing, proliferation and differentiation may occur, as desired. Often abundant extracellular matrix is produced which might cluster the cells together. Any suitable culture medium may be employed for the culturing, *e.g.* a culture medium as disclosed in WO 01/48147. In a preferred embodiment, this culture medium may be mixed to a desired extent with the buffer used in  
10 the formulation of a bone filler according to the invention.

It is preferred that an injectable bone filler according to the invention further comprises an osteoinductive factor. This factor will typically be incorporated in an amount in the range of 0.01 to 3 wt.%, based on the weight of the bone filler. Examples of suitable osteoinductive factors include  
15 growth factors such as BMP.

It has further been found advantageous to incorporate an angiogenic factor into the bone filler. An angiogenic factor may be used both in a bone filler that does not comprise cells, and in a bone filler that does. An angiogenic factor will typically be incorporated in an amount in the range of 0.01 to  
20 3 wt.%, based on the weight of the bone filler. Examples of suitable osteoinductive factors include growth factors such as FGF, VEGF, and PDGF.

It will be understood that the invention also encompasses a syringe having a needle and a reservoir wherein the reservoir contains a bone filler as described above. It will furthermore be understood that the syringe is to be  
25 kept under sterile conditions.

Of course, the invention further also encompasses the use of a bone filler as described above in the repair of osseous defects, wherein the bone filler is introduced into the defect by injection.

The invention will now be further elucidated by the following, non-  
30 restrictive examples.

### Example I

Fourth passage goat bone marrow cells were seeded onto densely sintered hydroxyapatite granules with a size of 212 to 300 micrometers, in a concentration of 200,000 cells per 200 milligram of hydroxyapatite. The cells were grown on the scaffold for 7 days in osteogenic culture medium comprising alpha-MEM, 15% foetal bovine serum, 0.2mM ascorbic acid-2-phosphate, 2mM L-glutamine, 10nM dexamethasone, 10mM beta-glycerophosphate and penicillin/streptomycin. The cell-coated granulate was subsequently mixed with a 3% alginate gel in PBS (sodium salt alginic acid, high viscosity, Sigma A7128) in a ratio of 58% alginate gel and 42% cell-coated hydroxyapatite (w/w). This paste was then subcutaneously implanted in nude mice (HsdCP:NMRI-nu, Harlan). After 4 weeks, the samples were retrieved and examined histologically.

A comparative study was performed wherein a paste was implanted, which was obtained by combining the three components hydroxyapatite granules, a cell suspension and an alginate gel (as described above) just prior to implantation, after which histology was performed 4 weeks post-operatively.

With both experiments, histological evaluation revealed that a fibrous tissue surrounded the implanted material paste. No signs of an inflammatory reaction could be observed, nor could histological differences be observed between implantation of the paste in mice or rats. At the periphery of the implant, early stages of tissue ingrowth and blood vessel formation were seen. Figure 1 shows the tissue reaction around hydroxyapatite granulate mixed with alginate gel after 4 weeks of implantation in Fischer rats. Note the fibrous tissue encapsulation and the absence of an inflammatory reaction.

From this study, it can be concluded that hydroxyapatite granulate, coated or combined with bone marrow cells and mixed with an injectable carrier such as alginate, results in a biocompatible injectable bone filler.

### Example II

PBS and algenic acid were mixed with a Braun multimixer or a blender. It is not preferred to do this with a normal mixer, because the alginate may agglomerate. Mix for 30 seconds with blender then 5 seconds by  
5 hand to prevent the alginate agglomerates sticking at the wall, then mix for another 30 sec. Mixing will cause a lot of air bubbles in the resulting gel. It is possible to suck these out of the gel with a vacuum furnace/pump twice for 5 seconds. Because of this, some water will vaporise. The amount of lost is  $\pm 0.16\%$  (this depends of course on the surface where the vaporization can take  
10 place).

In the gel thus obtained, hydroxyapatite particles (HA) were introduced through mixing. Different amounts of hydroxyapatite particles, as well as different sizes of hydroxyapatite apatite were studied and evaluated for injectability.

15 The injectability tests were performed with a Geniaplex syringe of 50 ml from the company Genia. If not mentioned different the outlet is a threated luer hub (code 109302, Genia). The syringe was fixed and the piston was connected with the loadcell of the tensile bench. The speed of testing is 75 mm/min. This speed was chosen, because it is more or less a normal speed of  
20 manual-injection. Every result is the average of a triple test. The Max (average) is the average of the 5 maximum tensile force points.

The following tables show the results achieved.

25 Table I: amount of HA (particle size 212-300  $\mu\text{m}$ , dense) v force for injection through needle of 2.2 mm diameter

Amount of HA in gel (wt.%)	Force needed for injection (N)
35	15.4
43	21.12
48	57.3

Table II: particle size of HA (in identical amounts) v force for injection through needle of 2.2 mm diameter

Particle size of HA ( $\mu\text{m}$ )	Surface of particles	Force needed for injection (N)
212-300	Rough	70.5
212-300	Dense/smooth	29.8
300-500	Dense/smooth	28.1

Table III: length of needle (diameter 2.2 mm) v force of injection

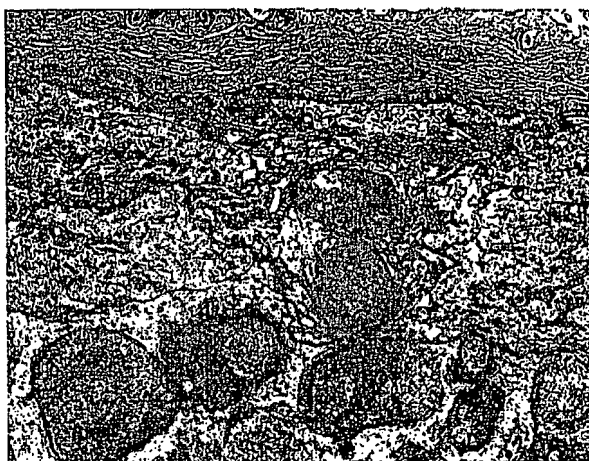
Length of needle (cm)	Force needed for injection (N)
0	78.8
0.5	85.4
1	89.8
2	92
3.5	107.2
5	139.4

Claims

1.           Injectable bone filler comprising calcium salt particles, an organic binder having an affinity for calcium salt, cells chosen from the group of stem cells, osteogenic cells, and osteoprogenitor cells, and a pharmaceutically acceptable buffer.
- 5   2.           Bone filler according to claim 1, wherein the particles are of a calcium salt are chosen from the group of calcium phosphates, monetite, brushite, (CaHPO<sub>4</sub>), calcium pyrophosphate, calcium carbonate, and combinations thereof.
3.           Bone filler according to claim 2, wherein the calcium salt is  
10 hydroxyapatite,  $\beta$ -calcium phosphate, and combinations thereof.
4.           Bone filler according to claim 3, wherein the particles have a diameter in the range of 100 to 600  $\mu$ m, preferably 200 to 400  $\mu$ m.
5.           Bone filler according to any of the preceding claims, wherein the binder is chosen from the group of alginates, dextrans, cellulose, cellulose  
15 derivatives, plasma, biogenic binders, hyaluronic acid, and combinations thereof.
6.           Bone filler according to claim 5, wherein the binder is chosen from the group of hyaluronic acid, sodium alginate, sodium carboxymethyl cellulose, dextran, fibrin glue, and transglutaminase.
7.           Bone filler according to claim 6, wherein the binder is sodium  
20 alginate.
8.           Bone filler according to any of the preceding claims, wherein the binder is present in an amount of from 0.5 to 10 wt.%, preferably from 3 to 7 wt.%, based on the weight of the bone filler.
9.           Bone filler according to any of the preceding claims, wherein the  
25 buffer is phosphate buffer saline (PBS).
10.          Bone filler according to any of the preceding claims having a solids content of 30-70, preferably 40-60 wt.%.

11. Bone filler according to any of the preceding claims having a viscosity between 30,000 and 100,000 centipoises.
12. Bone filler according to any of the preceding claims further comprising an angiogenic factor.
- 5 13. Bone filler according to any of the preceding claims, wherein the cells are present in seeded form onto the calcium salt particles.
14. Bone filler according to any of the preceding claims further comprising an osteoinductive factor.
15. Syringe comprising a needle and a reservoir, which reservoir  
10 comprises an injectable bone filler according to any of the preceding claims.
16. Syringe according to claim 15, wherein the needle has a length between 5 and 20 mm, and a diameter between 2 and 5 mm.
17. Method for preparing an injectable bone filler according to any of the claims 1-14, comprising mixing the binder and the buffer to prepare a gel,  
15 adding the calcium salt particles to the gel, and homogenizing to obtain the bone filler.
18. Method according to claim 17, in which cells are seeded onto the calcium salt particles before they are added to the gel, or wherein cells are introduced after combining calcium salt particles and the gel, the cells being  
20 chosen from the group of stem cells, osteogenic cells, and osteoprogenitor cells.
19. Method for repairing an osseous defect comprising injecting an injectable bone filler according to any of the claims 1-14 into the defect.

Figure 1



## INTERNATIONAL SEARCH REPORT

PCT/NL 02/00633

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A61L24/02 A61L27/38

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 287 341 B1 (REY CHRISTIAN ET AL) 11 September 2001 (2001-09-11) examples 6,13 claims 1-4,9-12	1-4,13, 15-17
X	WO 00 07639 A (BELL EUGENE; TISSUE ENG INC (US); SIOUSSAT TRACY M (US)) 17 February 2000 (2000-02-17) claims 1,4-7,9,13,18	1-5, 14-18
X	US 2001/014475 A1 (FINK DAVID J ET AL) 16 August 2001 (2001-08-16) claims 4-6,11,24,26	1-18

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the International filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the International filing date but later than the priority date claimed

- \*T\* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

Date of the actual completion of the International search

13 January 2003

Date of mailing of the International search report

22/01/2003

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Authorized officer

Heck, G



FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

Continuation of Box I.1

Although claim 19 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

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Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/NL 02/00633

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: —  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

PCT/NL 02/00633

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 6287341	B1	11-09-2001	US 2002136696 A1	26-09-2002
			US 6214368 B1	10-04-2001
			US 5676976 A	14-10-1997
			AU 742748 B2	10-01-2002
			AU 4674697 A	11-05-1998
			EP 0936929 A2	25-08-1999
			JP 2001502215 T	20-02-2001
			WO 9816268 A2	23-04-1998
			US 6139578 A	31-10-2000
			US 6277151 B1	21-08-2001
			US 6331312 B1	18-12-2001
			US 2002155167 A1	24-10-2002
			US 2002155137 A1	24-10-2002
			US 6117456 A	12-09-2000
			US 6132463 A	17-10-2000
			US 6027742 A	22-02-2000
			AU 711326 B2	14-10-1999
			AU 5865996 A	29-11-1996
			BR 9608409 A	17-08-1999
			CA 2220306 A1	21-11-1996
			CN 1184454 A	10-06-1998
			CZ 9703626 A3	13-05-1998
			EP 0825963 A1	04-03-1998
			FI 974210 A	09-01-1998
			HU 9901130 A2	28-04-2001
			JP 11511722 T	12-10-1999
			NO 975247 A	09-01-1998
			NZ 308816 A	29-09-1999
			PL 323448 A1	30-03-1998
			TR 9701392 T2	21-07-1998
			WO 9636562 A1	21-11-1996
			US 5650176 A	22-07-1997
			US 5683461 A	04-11-1997
WO 0007639	A	17-02-2000	AU 5671199 A	28-02-2000
			EP 1102603 A1	30-05-2001
			WO 0007639 A1	17-02-2000
			US 2002055143 A1	09-05-2002
US 2001014475	A1	16-08-2001	US 6378527 B1	30-04-2002
			US 2001051834 A1	13-12-2001
			AU 3626700 A	09-10-2000
			EP 1171057 A1	16-01-2002
			WO 0056251 A1	28-09-2000
			US 2002123142 A1	05-09-2002
			US 2002133235 A1	19-09-2002
US 2002012705 A1	31-01-2002			